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Histomorphometric analysis of mature female Japanese quail (*Coturnix coturnix japonica*) stomach

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ABSTRACT

The study analyzed the histomorphometry of the mature female Japanese quail (*Coturnix coturnix japonica*) stomach with the aid of ImageJ software. The different histological parts were identified using a compound microscope. Five mature laying female Japanese quail were collected and necropsied. The digestive organs, particularly proventriculus and gizzard, were collected and processed for tissue staining. Histological identification and measurement of thickness and depth of various structures were subsequently performed. Comparable to other avian species, the proventriculus was comprised of four layers: thin tunica serosa (22.69 µm), tunica muscularis (235.07 µm) with outer longitudinal and inner circular smooth muscle layers, thick tunica submucosa (2,164.37 µm) containing glands, and innermost tunica mucosa (553.42 µm) with papillae. The gizzard was characterized by four tunics: thin tunica serosa (60.44 µm), thick tunica muscularis (1,480.07 µm), tunica submucosa (112.25 µm), and tunica mucosa (456.15 µm) where the glands, crypts, and koilin can be found. The findings suggest that the histology of proventriculus and gizzard of the Japanese quail have no remarkable difference compared to other poultry species. However, the histomorphometry of the organs examined had remarkable variations as compared to other avians.

Keywords

gizzard; histomorphometry; Japanese quail; proventriculus

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Abbreviations

µm: micrometer
H & E: hematoxylin and eosin

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The poultry industry is one of the largest animal sectors in agriculture [1] and has rapidly evolved over the past 100 years from backyard household production to highly sophisticated commercial production units [2]. Nowadays, quails have gained particular attention because of their unique characteristics and as an alternative to chicken and duck eggs and meat. Quails sexually mature rapidly, have shorter incubation periods, are fast-growing and efficient food converters and prolific egg producers, therefore making them the most suitable and effective poultry [3]. Even though they are the smallest birds used for commercial production, quail production is still economically viable and technically feasible, in addition to its resistance to various diseases [4]. They have attained economic importance in the poultry industry through the unique flavor of their eggs and meat [5].

An ideal functioning digestive system is required as commercial poultry breeds are expected to efficiently utilize feeds [6]. The most active part of the avian digestive system is the stomach [7]. In birds, the stomach is divided into two distinct parts [8], the glandular portion (proventriculus) and the muscular portion (ventriculus or gizzard) [7].

The proventriculus or pars glandularis is the cranial compartment of the avian stomach which is functionally similar to the mammalian stomach. It is a relatively small tubular organ and is elliptical [9–12]. Grossly, it is situated caudal to the crop and connected to the gizzard [12,13]. It is responsible for the secretion of pepsinogen, hydrochloric acid (HCL), and the zymogenic component of gastric juice that is needed for the digestion of feeds [7–11,14].

The gizzard or the ventriculus is the muscular compartment of the avian stomach and incomparable to the mammalian stomach [9]. It is small and round in shape with tapering ends situated caudal to the proventriculus and relatively located between the lobes of the kidney and partly behind the left lobe of the liver [7,15]. In granivores, insectivores, and herbivores, the ventriculus is well-developed and distinct from the proventriculus [9]. This mechanical stomach is responsible for providing a suitable environment for the physical and chemical reduction of the bird's intricate nutritional diet [9,10,16].

Currently, there are limited researches on Japanese quail that can help us understand its microscopic anatomy. Many researchers prefer to use other species of birds such as chicken, ducks, and turkey due to the ease of collecting and visualizing their organs. Histomorphometric studies on the organs of Japanese quail are not given enough emphasis as compared to their egg and meat production aspects and traits. Hence, in the present study, histologic parts of the quail stomach were identified and measured. In addition, the study used mature female Japanese quails (more than six weeks of age) raised in the backyard for egg production, and no attempt was performed in determining the genetic purity of the quail or associate the management practices being implemented in the farm in the evaluation of the organ.

Proventriculus

Figure 1 shows the different histological parts of the proventriculus, particularly the four layers of the organ. These layers were also documented in various species of birds [7,17-21]. The tunica mucosa is the innermost layer consisted of numerous microscopic invaginating folds that varied in size and height, and arranged concentrically around the single duct that opens in the lumen [10,11,20,22,23].

The surface epithelium was a simple columnar type with cells having vesicular nuclei located near the basement and relatively pale staining cytoplasm (Figure 1 – upper left and upper right) that produce mucous secretion [7,10,11,17,18,20,22,24]. The lamina propria was constructed by loose connective tissue and blood vessels (Figure 1 – upper left and upper right). It is also constructed by lymphatic infiltration and tissues; the numerous blood vessels around the glandular epithelium were capillaries [12,18–20,22,24]. The lamina also extended inside the mucosal folds and contained simple tubular glands that opened into the lumen of the organ (Figure 1 – upper left) that produce mucous secretions [7,11].

The inner surface of the proventriculus displayed raised low and wide papillae on the lumen that serve as exit ducts of the composite gland for protein digestion and secretion of digestive juices [18,19]. Functionally, the tunica mucosa serves as a railway to the lumen of the proventriculus to convey the essential enzymes and secretions that are important in the digestion of feeds as well as the protection and absorption of nutrients [9].

The tunica submucosa comprised the bulk of the proventriculus since it catered the proventricular lobes and glands which formed the utmost thickness of the proventricular wall (Figure 1 – upper left). Similar to previous studies [11,13,17,18,22,23], the compound branched tubuloalveolar proventricular glands were multilobular in structure and arranged in pyramidal or conical shape (Figure 1 – upper left). The glands are characterized by oval, rounded, hexagonal, elongated, conical, or polymorphic lobules and are divided by a thin perilobular areolar connective sheath composed of fibroblast and smooth muscle fibers rich in blood vessels. The glands contain numerous secretary alveoli or tubules that open together in a wide central cavity, and ducts from several lobules merge together to form a short main duct that connects to the mucosal

papillae and opens in the lumen of the proventriculus [10,11,17–20,23,24].

The secretory cells forming the simple cuboidal epithelium were oriented obliquely to the long axis of the tubules and were divided by a narrow space giving a serrated appearance (Figure 1 – upper left and upper middle). Such oxyntico-peptic cells possess regularly large and round nuclei which are located close to the basement of the cell. These cells are capable of secreting both hydrochloric acid and pepsinogen which aid in the better digestion and breakdown of feeds [10,18,20,23,24].

The tunica muscularis consisted of two layers with inner circular and outer longitudinal smooth muscle fibers (Figure 1 – upper left and upper middle). As observed in previous studies [7,10,11,17-20,24], the inner circular smooth muscle fiber was thicker and characterized by round basic cells scattered throughout the muscle bundles, while the thin outer longitudinal muscle was characterized by flat basic cells also scattered throughout. The tunica muscularis is accountable for the back-and-forth motility of feeds in the gizzard and proventriculus [9].

The tunica serosa is the outermost layer of the proventriculus consisting of smooth muscle fibers. including dense connective tissue in which nerves and blood vessels are distributed can also be found in this layer [7,11,18,19].

Table 1 shows the measurements of the microscopic parts of the quail proventriculus including its four main layers: the tunica mucosa, the tunica submucosa which is the thickest due to the numerous proventricular glands found in this layer, the tunica muscularis, and the tunica serosa which is the thinnest. It was noted that the tunica muscularis at the posterior section of the organ is relatively thicker than the anterior and middle sections. This may be attributed to the gradual transition of the organ as it connects to the muscularly structured gizzard.

In the study on striated scope owl [7], the mean thickness of the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa was about five,

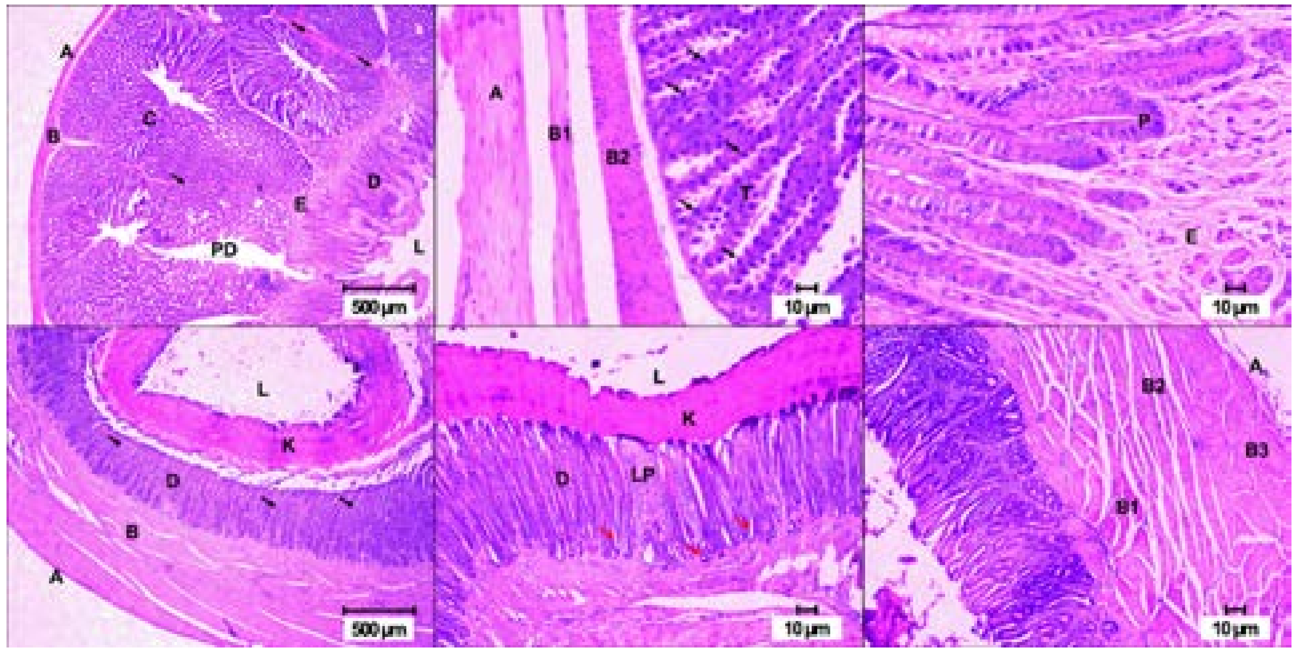

Fig 1.
Transverse sections of the proventriculus (upper) and gizzard (lower) mature female Japanese quail (*C. coturnix japonica*) stained with H & E at 40x magnification.
The proventriculus: (A) tunica serosa, (B) tunica muscularis, (B1) tunica muscularis (outer longitudinal), (B2) tunica muscularis (inner circular), (C) tunica submucosa, (T) tubuloalveolar proventricular glands in the tunica submucosa, (D) tunica mucosa, (E) lamina propria, (P) papillae, (L) lumen, (PD) primary duct, and black arrows are the septa (upper left), secretory cells (upper middle). The gizzard: (A) tunica serosa, (B) tunica muscularis, (B1) inner oblique muscle layer of the tunica muscularis, (B2) circular muscle layer, and (B3) outer longitudinal muscle layer, (C) tunica submucosa, (D) tunica mucosa, (K) koilin, (L) lumen, (LP) lamina propria, and black arrows are the crypts (lower left), and red arrows are the glands (lower middle).

six, 17, and five times, respectively, thicker than in quail. While the current study revealed that the depth of proventricular gland in a mature female Japanese quail is about 1,288.90 µm in diameter and the tunica mucosa is 553.42 µm thick, the proventricular gland height of a 45-day old male Japanese quail is 9.60 µm and the thickness of the mucosal surface is 28.58 µm [23].

Gizzard

The gizzard or the ventriculus is unique in birds among other vertebrates. It is the muscular compartment of the avian stomach and incomparable to the mammalian stomach [9]. It is small and round in shape with tapering ends situated caudal to the proventriculus and relatively located between the lobes of the kidney and partly behind the left lobe of the liver [7,15]. This mechanical stomach is responsible for providing a suitable environment for the physical and chemical reduction of the bird's intricate nutritional diet [9,10,16].

Figure 1 (lower left) shows the different histological parts of the gizzard. The histologic structure of the gizzard wall in quail was composed of four layers or tunics similar to what was found in the proventriculus. These four layers were (innermost to outermost) the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. In addition, a keratinized membrane covering called koilin was also present.

The tunica mucosa was the innermost layer consisting of invaginating long mucosal folds embedded in the lamina propria (Figure 1 – lower left and lower middle). The invaginating folds are the so-called gastric pits and at the base of these folds are the crypts [7,19,25]. These gastric pits range up to the glandular layer and subjugate the majority thickness of the mucosa. Simple tubular glands open into the shallow crypts. These straight tubular glands are also bounded by dense connective tissue with collagen and are filled in their upper parts by a material that becomes continuous with the dense layer overlying the mucosa [16]. The gastric glands are also situated in the tunica mucosa and are lined principally by chief cells located in the lower parts of the glands which are cuboidal in shape whose nuclei are more or less rounded in outline. These cells produce a protein-rich secretion [9,16,25]. The basal cells are few as compared to the chief cells. They occur by pair and are located in the depth of the gland. These cells have a large round pale nucleus, complex nucleolus, and pale staining cytoplasm and have no granules. Since they are situated in the mitotic zone of the gland, it could be possible that they are stem cells. There were intermediate cells also found in the deep part of the glands [16]. The gizzard glands are essential in enzyme and cell secretions that are important in the digestion of feeds as well as protection and absorption of nutrients [9].

There were more lamina propria constructed by a loose connective tissue separating the pits between glands. The surface epithelium was composed of simple low columnar cells to simple cuboidal cells with round vesicular basal nuclei. While the epithelium and the lamina propria were both present, there was no muscularis mucosa between the lamina propria and the underlying tunica submucosa (Figure 1 – lower left and lower middle). Previous studies [7,10,12,23,25] also reported the same observations.

The koilin was a thick horny layer of keratinoid that served as a covering membrane of the tunica mucosa (Figure 1 – lower left and lower middle). This layer with a relatively wavy pattern is formed by the secretion of gizzard glands and consists of scaffolding of interrelating vertical rods and horizontal matrix [9,13,14]. The superficial koilin is less dense than the deeper koilin trapped within the glandular epithelium [10]. The sandpaper-like texture of the koilin acts as a grinding surface of the feeds to increase their surface area, to promote better gastric proteolysis, and also to serve as a protection layer [8,16].

The tunica submucosa of the gizzard was indistinguishable from the lamina propria (Figure 1 – lower left). However, it was bulky and composed of abundant loose connective tissue that was richly supplied with blood vessels and nerves as similarly observed in previous studies [7, 21,23].

The tunica muscularis was structured by a well-developed and strongly thick, three smooth muscle layers namely, the outer longitudinal, the middle circular and the inner oblique (Figure 1 – lower left and lower right). This layer comprised the bulk of the organ to justify its major role in grinding and macerating the complex diet of avian species. These results were similar to those of Ahmed et al. [23], Kadhim et al. [10], and Al-Saffar & Al-Samawy [21] but contrary to the findings in coot bird [17] and red-capped cardinal [12]. The muscle bundles are also interposed with bands of connective tissues rich in blood vessels and nervous ganglions of mioenteric or the auerbach plexus [12]. The tunica muscularis is accountable for the strong contractions of gizzard in triturating the ingesta to decrease the size of the feed. It is also responsible for the motility of feeds and back and forth in the proventriculus and for propelling the feeds into the small intestine by muscle contraction [8,16].

The tunica serosa was the outermost layer, and, similar to previous reports [7,19,21], mainly consisting of dense loose connective tissue lined by a simple squamous mesothelium, blood vessels, lymphatic vessels, and nerves. In addition, Catroxo et al. [12] mentioned that it is also constituted by adipose cells and nervous elements of a plexus.

Table 1 shows the measurements of the different microscopic parts of the quail gizzard. The organ is composed of four main layers, the tunica mucosa, the tunica submucosa, the tunica muscularis which was the thickest, and the tunica serosa which was the thinnest. It can be noted that the thickness of the tunica muscularis decreases as it transitions to the duodenum of the small intestine.

Al-Saffar & Al-Samawy [7] described the mean thickness of the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa, which was about three, 15, three, and two times, respectively, thicker than the corresponding layer in quail. Moreover, in a study on a 45-day old male Japanese quail [23], the koilin layer was 336.74 µm in thickness which is slightly similar to the measurement obtained in this study in mature female Japanese quail (about 318.36 µm). However, the development of this keratinized layer of the gizzard varies among other avian species. In granivores, insectivores, and herbivores, the gizzard is well-developed and koilin is present while, in carnivores and piscivores, the gizzard is poorly developed and there is an absence of koilin [9,26]. Though koilin was reported in various species of birds [10,17,19,21,23,25], it was not documented as a histologic part in striated scope owl, krestel, and Eurasian sparrowhawk [7].

Based on the findings of the study, the histology of the proventriculus and gizzard of the Japanese quail revealed no remarkable difference compared to the other poultry species, hence, their stomach is almost precisely similar to that of the chicken. However, the histomorphometry of the organs examined had remarkable variations as compared to other avians.

Materials & Methods

Collection of animals and preparation of samples

A total of five mature female Japanese quails were collected from a backyard raiser. The birds were carefully euthanized through cervical dislocation and immediately followed by decapitation [27-29]. The organs were collected and washed using a physiologic saline solution. The tissues were cross-sectionally cut into 1 to 3 cm tissue blocks and preserved in 10 percent buffered formalin. The fixed tissue samples were processed for paraffin technique and stained with H & E stain.

Microscopic examination of tissues

The prepared tissue slides were examined using a compound digital microscope (Olympus CX23, Tokyo, Japan). The major histological parts of the proventriculus such as the layers (tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa), the proventricular glands, and plicae were identified, photographed, and described. Likewise, the microscopic

Table 1.
Histomorphometry of the histological parts of the mature female Japanese quail (*C. coturnix japonica*) proventriculus and gizzard.

Histologic Parts	Proventriculus		Gizzard	
	Thickness (µm)	Diameter (µm)	Thickness (µm)	Depth (µm)
Tunica mucosa	553.42 ± 229.97	-	456.15 ± 104.55	-
Lamina propria	145.58 ± 64.18	-	-	-
Koilin	-	-	318.63 ± 127.66	-
Crypt	-	-	-	226.67 ± 70.87
Tunica submucosa	2,164.37 ± 358.98	-	112.25 ± 69.19	-
Proventricular gland	-	1288.90 ± 402.55	-	-
Papillae	-	431.38 ± 175.74	-	-
Tunica muscularis	235.07 ± 264.63	-	1,480.07 ± 739.43	-
Tunica serosa	22.69 ± 9.11	-	60.44 ± 30.29	-

The data were presented as mean ± standard deviation (SD) of the examined histologic parts (3 to 6 microscope fields of view per section: upper, middle, and lower sections of the proventriculus and gizzard)

structures of the ventriculus such as the layers (the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa), the glands, and the keratinized layer (koilin) were also identified, photographed, and described. The histomorphometry of these different histological parts was measured using a compound digital microscope (Olympus CX23, Tokyo, Japan) and the images obtained were measured for thickness or diameter in µm using ImageJ software (Java-based, version 1.52v, LOCI University of Wisconsin-Madison) [30].

Statistical analysis

Each organ per bird was measured in triplicate. The measurement data obtained from the histological parts of the randomly selected proventriculus and gizzard tissue sections from the samples were presented as mean ± standard deviation (SD).

Authors' Contributions

F.B.R.M. conceptualized the research, initiated the writing of research outline and the final manuscript, examined the tissues and performed the statistical analyses and interpretation of results, and arranged and reviewed all parts of the manuscript, including formatting. N.A.M.D.C. prepared the samples for tissue processing and staining, examined the tissue slides and performed the image capturing and measurement of histologic parts, and wrote the materials and methods and some parts of the results and discussion. M.B.S.S. examined the captured images before measurement, identified the histologic parts of all tissue samples, and wrote some parts of the results and discussion.

Competing Interests

The authors declare that they have no competing interests.

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